From Concept to Practice: A Comprehensive Review of Two Decades of DNA Barcoding

Almuthanna k. Alkaraki[1], Abrar Rashdan[1], Omar Gammoh[2], Esam Qnais[3], Abdelrahim Alqudah[4], Mohammad B. Alsliman[1], Alaa A. A. Aljabali[5]

[1]Department of Biological Sciences, Faculty of Science, Yarmouk University, Irbid 21163, Jordan; alkaraki@yu.edu.jo; abrar.rashdan@yu.edu.jo; m.b.alsliman@gmail.com
[2]Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmacy, Yarmouk University, PO BOX 566, Irbid, 21163, Jordan; omar.gammoh@yu.edu.jo
[3]Department of Biology and Biotechnology, Faculty of Science, The Hashemite University, Zarqa, Jordan; esamqn@hu.edu.jo
[4]Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmaceutical Sciences, The Hashemite University, Zarqa 13133, Jordan; abdelrahim@hu.edu.jo
[5]Faculty of Pharmacy, Department of Pharmaceutics & Pharmaceutical Technology, Yarmouk University, Irbid 21163, Jordan; alaaj@yu.edu.jo
Received: 4 Oct. 2024, Accepted: 25 Oct. 2024, Available Online: 27 Oct. 2024

Abstract

DNA barcoding has significantly revolutionized species identification and conservation efforts. This review delves into the applications, challenges, and integration with other complementary molecular and computational tools. High-throughput sequencing (HTS) has enabled the recruitment of different genetic markers (Barcodes) such as cytochrome c oxidaseI (MT-*CO*1) in animals, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*), and maturase K (*matK*) in plants. Combining these markers with machine-learning algorithms, such as random forests and Convolutional Neural Networks, has led to significant advancements in the accuracy and efficiency of conservation efforts. DNAbarcoding has significantly improved our biological knowledge by identifying cryptic species and resolving taxonomic issues. DNA barcoding has been used in forensics, conservation, andagriculture. And also essential in food identification and product authentication. In this critical era of biodiversity, further development and application of DNA barcoding are crucial for creating effective conservation strategies. The International Barcode of Life (iBOL) aims to standardize and expand DNA barcoding databases, targeting the update of five million specimens from 500,000 species by 2026. The Barcode of Life Data Systems (BOLD) has grown from 5,000 to over 10 million sequences by 2021, demonstrating rapid progress. However, challenges persist, including the limited taxonomic and geographical coverage. Moreover, this scope raises ethical concerns, including biopiracy, which the Nagoya Protocol addresses by emphasizing responsible scientific progress.

Keywords: DNA Barcoding, Biodiversity, Taxonomy, Molecular Markers, Species Identification.

DNA barcoding helps to identify species in ecosystems and has many applications. Scientists have recruited it in ecology, evolution, conservation, sample identifications, and products authentication. It also aids in forensic investigations and biodiversity surveys. Advancements in DNA barcoding methods, such as mini-barcodes and multispecies

coalescent species delimitation, have enhanced their utility. DNA barcoding is a fast and reliable tool for species identification, taxonomic classification, biodiversity studies, molecular ecology, and population genetics research (Che et al., 2012; Fasullo & Dolan, 2022). DNA barcoding allows for a standard and effective identification of species in different organisms and ecosystems (Fasullo & Dolan, 2022; Y. Liu et al., 2021; Vences, Miralles, & DeSalle, 2024). DNA barcoding has been used in other applications, such as studying toxicological effects, tracking cell lineages, high-throughput screening for biomolecules, differentiation among plant species, and finding agriculturally important insects (Şapcı Selamoğlu, 2022; Vuataz et al., 2024). Moreover, DNA barcoding can distinguish between closely related species and map the spread of plant roots when studying invasive plants (Gostel & Kress, 2022; Nath et al., 2024).

DNA barcoding is a simple method for identifying organisms using short standardized DNA segments (P. D. Hebert et al., 2003). It utilizes an organism's DNA sequence, similar to how a supermarket scanner reads an item's barcode, thus making each species unique (Hollingsworth, Graham, & Little, 2011). Usually, the barcode region varies between 400 to 800 base pairs long (Kress, 2017). Samples come from many types of specimens found in the wild and could be collected from museums, zoos, gardens, and seed banks (Schindel & Miller, 2005). Once the barcode sequence is ready, it can be stored in a database of the reference sequences. It works as a specimen collection number but identifies the species found by the researcher (S. Ratnasingham & P. D. N. Hebert, 2007). DNA barcoding can speed up the identification of known species and help describe new species (Packer, Gibbs, Sheffield, & Hanner, 2009; Valentini, Pompanon, & Taberlet, 2009).

DNA Barcoding was first introduced by Herbert et al.(P. D. Hebert, A. Cywinska, S. L. Ball, & J. R. deWaard, 2003), who recruited a standardized DNA region for species identification. Herbert proposed using the mitochondrial gene, cytochrome c oxidase I (*MT-CO1*), as a DNA barcode for animal identification. This gene was chosen because it evolves slowly within species but quickly enough between species, making it suitable for species-level identification in many animal taxa. DNA barcoding aims to resolve the challenges faced in traditional taxonomy. Morphology-based identification is considered time-consuming and requires expert knowledge and high experience. DNA barcoding is a faster, more reliable, and more accurate method for species identification (Hobern, 2021; A. Miralles, N. Puillandre, & M. Vences, 2024).
DNA barcoding faced significant challenges and criticisms, particularly regarding plant and fungal identification, early criticisms highlighted the limitations of plant and fungal identification using the universal *COI* gene. To address these limitations, researchers have adapted a combination of chloroplast candidate regions as barcodes for plants (plastid *rbcL*, *matK*, and *trnH-psbA*) along with nuclear internal transcribed spacer *ITS* (Bammer et al., 2020; Guo, Yuan, Tao, Cai, & Zhang, 2022; Kang, Deng, Zang, & Long, 2017; Kress, 2017), while nuclear *ITS* was recruited in fungi (Duan, Wang, Zeng, Guo, & Zhou, 2019; Mahmoud & Zaher, 2015). These efforts not only proposed a solution for the plant and fungal identification dilemma but also introduced a reform to the original approach. However, adopting new DNA segments as barcodes adds complexity to the standardization efforts (Fazekas et al., 2009; Spooner, 2009). Although there are differences in the choice of target DNA markers among researchers and challenges in generating barcodes for some taxa, DNA barcoding has become a standard identification tool, enabling the identification of many unidentified or cryptic species (China Plant et al., 2011).

In 2004, the Consortium for the Barcode of Life (CBOL) was launched as an international organization focused on

developing DNA barcoding as a global taxonomy standard, fostering a research alliance among more than 120 organizations from 45 countries (Marshall, 2005). The vast amount of data generated from this collaboration led to the development of the Barcode of Life Data System (BOLD) (S. Ratnasingham & P. D. Hebert, 2007). BOLD is a publicly accessible database system that efficiently compiles sequences that meet the standards required for designating barcodes in a global sequence library (Figure 1). This allows researchers to acquire barcodes and compare them with sequences from unidentified samples, thereby facilitating the identification of new species, and supporting the storage and dissemination of DNA Barcodes. BOLD currently houses more than 12 million DNA Barcodes (Ratnasingham, Wei, Chan, Agda, Agda, Ballesteros-Mejia, Boutou, El Bastami, Ma, Manjunath, et al., 2024; Sujeevan & Hebert, 2007). Recent advances in high-throughput sequencing (HTS) technologies have enhanced the power of DNA barcoding. According to the BOLD website, this public record database has published more than two million *COI* Sequence records from BOLD and GenBank, representing more than 50,000 confirmed species, along with a total of more than 60,000 Interim Species (S. Ratnasingham & P. D. N. Hebert, 2007; Stoeckle & Hebert, 2008; Sujeevan & Hebert, 2007). This indicates the emergence of a revolutionary taxonomic approach, which has gained significant traction in the field of taxonomy. In addition to taxonomic research using DNA barcoding technology, DNA barcoding applications have been expanded to fields such as pest control, bio-management, and biosecurity. This development is driven by the need for rapid, accurate, and efficient species identification, which is critical in these fields (Thiele et al., 2021; Vernygora, Sperling, & Dupuis, 2024).

Additionally, metabarcoding, which allows the simultaneous identification of multiple species from complex environmental samples, has enhanced biodiversity and biomonitoring studies. Metabarcoding can provide a more comprehensive assessment of ecosystems, which is important for measuring the impact of climate change and habitat degradation. Metabarcoding along with HTS technology has improved the study of the genomic parts of entire communities in complex environmental samples, helping biodiversity and biomonitoring studies (McDonald et al., 2023; Aurélien Miralles, Nicolas Puillandre, & Miguel Vences, 2024). Given the current threats to biodiversity and high rates of extinction, such ecological studies are important for measuring the impacts of climate change, habitat loss, and ecosystem management and restoration (Belwal & Jadeja, 2024; Onoh, Ogunade, Owoeye, Awakessien, & Asomah, 2024; Wang et al., 2024). For instance, in biomonitoring and bioassessment programs, accurate and consistent taxon identification in bulk samples is vital for providing the data needed to make management decisions and protocols. However, traditional monitoring procedures cannot effectively provide the data required for such decisions (Dallas, 2021; Simaika et al., 2024). Metabarcoding finds more taxa per sample than the traditional sampling methods and accurately describes changes in community composition, improving the ability to detect ecosystem changes and dynamics and make decisions and protocols accordingly (Nørgaard et al., 2021; Tsuji et al., 2022)

DNA sequencing and metabarcoding are new methods for studying biodiversity and for improving biomonitoring. However, these methods have significant drawbacks and controversies that require further investigation. One issue is overconfidence in the data due to reliance on these technologies. Metabarcoding is biased by PCR amplification, sequencing errors, and possible non-target amplification. This can lead to incorrect results and misinterpretations of the community composition (Shelton et al., 2023). For example, rare taxa may go unnoticed

or be misidentified, making it difficult toevaluate ecological health.

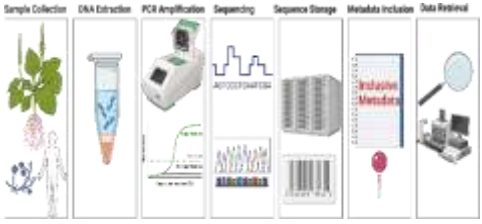

Figure 1. Overview of the DNA Barcode Database Workflow.

Another problem is that molecular analysis alone rarely captures the complexity of ecological systems. Many factors besides genomics affect biodiversity, including species interactions, life history, and environmental variability (Hakimzadeh et al., 2024). Although limited, traditional methods can provide valuable information through ecological knowledge, historical data, and thorough assessments, which molecular techniques may overshadow. Thus, a full picture of ecosystem health often requires combining traditional methods and molecular techniques, rather than relying on a single approach.

Practical challenges such as cost, access to technology, and the need for specialized skills to analyze genomic data also limit the widespread adoption of metabarcoding, especially in resource-limited settings (Keck et al., 2022). Without proper training and infrastructure, there is the risk of misapplying the technique or misinterpreting the results, leading to misguided conservation efforts. In summary, DNA sequencing and metabarcoding have significantly improved biodiversity studies. However, relying solely on these new techniques without considering ecological nuances or integrating older methods would overlook crucial insights, bias data interpretation, and complicate biodiversity management and conservation. Therefore, care is required in understanding how these technologies are viewed and used in ecological research and biodiversity monitoring.

New sequencing platforms offer faster results and lower costs. This allowed iBOL to start the seven-year BIOSCAN program (https://ibol.org/programs/bioscan/). Through an international partnership, BIOSCAN aims to create a barcode reference library for more than two million species from 2019 to 2026 (De-Kayne et al., 2021; de Medeiros et al., 2024; Satam et al., 2023). Barcode reference libraries will grow significantly, and metabarcoding of these specimens will provide useful data on thousands of ecosystems worldwide, and on the interactions between thousands of organisms within them. Metabarcoding and HTS technologies were initially considered threats to the barcoding approach (de Medeiros et al., 2024; R. Gwiazdowski, 2024). However, they also add value to the DNA barcoding.

DeSalle and Goldstein reviewed more than 3,700 peer-reviewed articles on DNA barcoding, published between 2003 and 2018. These studies have shown that DNA barcoding is useful in taxonomic studies. Their work predicted the wide use of DNA barcoding in future taxonomic research, highlighting diversity as a key idea and practice (DeSalle & Goldstein, 2019). Hebert et al. (2023) reported a strong increase in yearly DNA barcoding publications across various scientific journals, emphasizing the growing impact and the global use of this technique. Their analysis showed steady growth in research output, indicating the method's increasing influence in different areas of biology. Despite their widespread use, DNA barcoding poses several challenges. These include the need to expand reference libraries, technical issues with PCR amplification and sequence analysis, problems with preserving and interpreting variation between species, and ethical concerns regarding the privacy and ownership of genetic data. These challenges suggest that DNA barcoding requires further investigation. Future research should focus on improving DNA barcoding methods and applications (P. D. N. Hebert et al., 2018).

DNA barcoding which had likely started as a short-term solution to overcome technical

limitations when it was first proposed evolved to a modern molecular tool for various scientific uses (P. D. N. Hebert, A. Cywinska, S. L. Ball, & J. R. DeWaard, 2003). DNA barcoding allows for quick identification and listing of many different species. It is a useful tool that can greatly improve taxonomy for biodiversity conservation (P. D. N. Hebert et al., 2018). This review aims to enhance understanding of DNA barcoding while evaluating its current status and potential uses. We examined the successes and challenges found in the existing research to guide future work in this field (Pentinsaari, Ratnasingham, Miller, & Hebert, 2020). We identified knowledge gaps, including the need to improve DNA-barcoding techniques. Some upcoming uses of DNA barcoding include checking environmental health, identifying invasive species, and testing food authenticity. In the future, molecular taxonomy may be combined with other technologies, such as blockchain for secure data management and artificial intelligence, to further automate the analysis.

Table 1. SWOT analysis provides guidelines for internal and external factors that influence the potential of DNA barcoding. It should be highlighted that successful DNA barcoding relies on overcoming weaknesses and exploiting opportunities for further improvement and application.

Strengths	Weaknesses
1. Robust Precision in Various Conditions: DNA barcoding has been proven to be highly accurate in the face of even the most extreme environmental conditions.	1. Addressing Limited Species Coverage: This limitation will continue to be mitigated properly by expanding large-coverage reference databases.
2. Rapid Species Identification: The technique can rapidly identify species, useful in rapid cases of ecological studies or conservation activity.	2. Technical Constraints Overcome: Scientists are constantly trying to resolve technical constraints; ongoing research on the improvement of PCR amplification, sequencing, raw data processing and Data Deposition in GenBank.
3. Integral Biodiversity Conservation Tool: The utility of Biodiversity assessment and conservation exercise is proven at the global base, becoming a valuable parameter for global Conservation strategies.	3. Inter-Species Variation Management: Different approaches to address the problems posed by inter-species variation must be severely promoted, especially the utility of DNA barcoding.
4. Automated Processes Increasing Efficiency: Advancements allow automation, which increases the efficiency of large-scale analyses, decreases human error, and facilitates faster results.	4. Research Work in providing cost-effective alternatives and infrastructural solutions for delivering more access, and databases.
5. Progress Towards Standardization: There are now active efforts to standardize protocols, hence making even very different studies comparable.	5. Ethical considerations and solutions: Provisions are made to recognize ethical concerns, and discussions on issues of privacy and ownership are carried out as an integral part of good research practices to ensure responsible use.

Opportunities	Threats
1. Technology integration innovations: Building over and adding value to the DNA barcoding capacity through the leverage of high throughput technologies like Next Generation Sequencing.	1. Legal and Ethical Landscape: Negotiating future legal challenges and living up to the expectations of ever-changing ethical standards on responsible use.
2. Case Studies of Successful Integration: Specific details of examples of successful integrations conducted with other technologies to demonstrate possible advancements.	2. Data Security Protocols: Robust data security and keeping abreast of technological development to secure the information for genetic studies.
3. Global Database Expansion: Develop global reference databases for complete expansion together to support accurate species identification employing genetic diversity libraries.	3. Consequences of Misidentification: Real examples of consequences deriving from misidentification, underlining the importance of having correct reference databases.
4. Targeted Educational Strategies: Develop and deliver relevant and suitable educational and awareness programs targeted at researchers, policymakers, and the public to promote greater acceptability and appreciation.	4. Dynamic Technology Landscape: Keep tracking new technologies and their possible impact on DNA barcoding; be flexible as technology evolves.
5. Examples of Environmental Monitoring Projects that have Applied DNA Barcoding to Good Effect, Exemplifying Potential Impact.	5. Creative Funding Mechanisms: Creative ways of acquiring funds and innovative models to beat resource constraints for the sustenance of the search.

2. Methodology:

DNA barcoding has become an effective method for species identification, using short and specific DNA sequences (P. D. N. Hebert et al., 2018). The DNA barcoding process involves several important steps as shown in Figure 2, that are necessary to correctly identify a species (Bohmann et al., 2022). Research begins with the collection and storage of samples, which varies according to the type of organism. Appropriate storage methods are crucial, whether using ethanol, silica gel, or freezing to keep the DNA intact (Srivathsan, Nagarajan, & Meier, 2019). DNA extraction follows specific steps, depending on the sample type and target organism (Kristy Deiner et al., 2017). Common methods include CTAB extraction and phenol-chloroform purification, and many ready-made kits are available (Anslan et al., 2021). The quality and amount of extracted DNA greatly affect the success of later steps in the barcoding process (Braukmann et al., 2019).

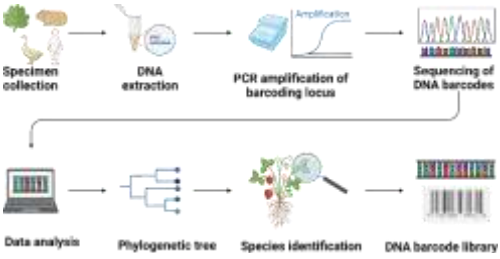

Figure 2. Schematic illustration of DNA barcoding from DNA isolation of the species under investigation to DNA extraction, PCR amplification of the barcoding locus, DNA sequencing for the DNA barcodes, data analysis, establishing the phylogenetic tree, species identification, and finally reaching the DNA barcode library. The image was created using Biorender.com.

The next critical step is PCR amplification of the target gene region

Primer selection is based on their effectiveness across multiple species, how specific they are, and the length of the DNA that should be amplified. Mitochondrial genes are frequently used to generate DNA barcodes in animals. This gene is useful because it varies significantly between animal species. In plants, scientists typically use a mix of chloroplast genes, *rbcL* and *matK*, along with the nuclear internal transcribed (ITS) spacer region (Che et al., 2012). Fungal studies often use internal transcribed spacer (ITS) regions (Xu, 2016). The process of DNA amplification often requires optimization of PCR conditions; Researchers may need to adjust annealing temperature and the number of cycles for different groups of organisms during amplification experiments. Sanger sequencing is often used for single specimens. Newer sequencing technologies have improved this step through the rapid processing of multiple samples. It is important to verify the quality of the sequence data at this stage (Fasullo & Dolan, 2022).

Computer software is recruited to clean up the sequences by trimming noisy sequences at the beginning and end of the chromatograms, removing low-quality sections, and eliminating mixed sequences. Obtained sequences (recommended not less than 400 bases) should be compared (aligned) with other sequences deposited earlier in a reference database, such as BOLD or NCBI nucleotide Entrez/GenBank, along with local alignment tools, such as NCBI BLAST. To assign species names, methods based on either genetic distance or character-based are recruited to draw phylogenetic evolutionary trees (Gao, Liu, Wang, Wei, & Han, 2019; Gostel & Kress, 2022). Finally, researchers examined the data verified their accuracy, and carefully checked how well the sequences matched, and where they fit into the tree of life as either identified species or newly recognized species. Taking into consideration where the species lives, its environment, and, if possible, its physical features, or other genetic data. The quality of species identification

depends on the completeness and accuracy of the selected reference databases.

The quality of the reference databases is very important. These databases require the identification of specimens by experts (based on classical taxonomical methods). Scientists are working to add more species and improve how they organize and standardize the data. They have also developed new ways to identify species using the distances between DNA sequences, specific DNA features, and computer programs that can learn patterns (Joly et al., 2014; Lahaye et al., 2008).

Therefore, it is important to choose databases with a higher number of entries, ensuring updating databases by depositing more sequences related to species from different groups and locations to these databases (Jalali, Ojha, & Venkatesan, 2015).

Although DNA barcoding techniques have significantly improved in recent years, some challenges persist. Selecting appropriate DNA regions for barcoding has been straightforward for many taxonomic groups. However, for certain groups such as protists, identifying universal DNA regions that work effectively across all species remains challenging. When designing and choosing primers, researchers must balance between them, to work with many species, while still being species-specific. They often use primers that are designed to target conserved sequences and can be slightly changed to match different target sequences. Researchers have created shorter barcodes for old or processed materials with damaged DNA (Joly et al., 2014).

In addition, Species can mix their DNA by breeding or by sharing genes over time. In newly split species, the DNA may not be sufficiently different. Some organisms have additional sets of chromosomes or mtDNA types within an individual. These issues make it difficult to distinguish between species by using DNA barcodes alone. Therefore, scientists often need to use other methods in addition to DNA barcoding (J. Liu et al., 2017; Y. Liu et al., 2021). Recent improvements have led to improvements in DNA-barcoding techniques. New sequencing methods can be used to analyze more samples faster and cheaper. This allows scientists to study multiple DNA markers in complex environmental samples routinely (Lyons, Sheridan, Tremmel, Miyano, & Sugano, 2017). New technologies that can read longer DNA sequences can assist with full-length barcodes or multiple regions of the DNA. This could provide better results for hard-to-analyze groups. Portable sequencing devices allow for DNA barcoding in the field; however, data quality and analysis remain challenging (Parveen, Gafner, Techen, Murch, & Khan, 2016).

Environmental DNA barcoding has significantly increased in recent years. Special methods are used to collect and extract DNA, considering how it breaks down in the environment. Scientists have combined DNA barcoding with whole-genome sequencing. This opens new avenues for exploring different DNA regions for barcoding (Shokralla et al., 2014). Although the basic concept of DNA barcoding remains the same, ongoing technological improvements make it more accurate, efficient, and useful for different groups of organisms and sample types. Many scientists believe that DNA barcoding is a key technique for studying species and biodiversity. As it combines new sequencing technologies and computer tools, its importance is likely to grow (Shokralla, Spall, Gibson, & Hajibabaei, 2012).

Since its discovery, the application of DNA barcoding has increased, and this technique has changed many areas of biological research and applications. This paper describes recent developments in these areas and their implications for molecular biologists, ecologists, and other professionals (Bezeng et al., 2017).

DNA barcoding has significantly improved biodiversity and conservation. The ability to quickly determine the identity of a species has transformed our capacity to catalog and monitor biodiversity. For example, a study of tropical arthropods (such as insects and spiders) using DNA barcoding showed that looking at physical features alone missed

approximately 40% of the species (P. D. N. Hebert et al., 2003). In oceans, DNA barcoding projects around the world have identified many new fish species, quickly adding to our knowledge of marine life (Bhattacharya et al., 2016; Ghosh, Bankura, & Das, 2016; Radulovici, Archambault, & Dufresne, 2010).

Research on environmental DNA (eDNA) has advanced the field of biodiversity monitoring. Deiner et al. (K. Deiner et al., 2017), found that eDNA metabarcoding detected 44% more species in river systems than traditional kick-net sampling. This non-invasive approach is useful for monitoring endangered species. Using only water samples, researchers have detected the presence of endangered Yangtze finless porpoises (Lim et al., 2016; Tolley-Jordan, Chadwick, & Triplett, 2023). DNA barcoding has contributed significantly to the field of conservation biology. Large surveys have identified new biodiversity hotspots, thus informing conservation strategies. This enhances authentication in herbal medicines and contributes to plant conservation. 59% of the tested herbal products contained DNA barcodes for plant species that were not listed on their labels. Some of these plants are endangered (Kress, García-Robledo, Uriarte, & Erickson, 2015; Newmaster, Grguric, Shanmughanandhan, Ramalingam, & Ragupathy, 2013).

DNA barcoding has also improved the forensics of wildlife crime. DNA barcoding helps identify illegal wildlife products and increases conviction rates for wildlife trafficking (Arenas et al., 2017; Elkins & Zeller, 2021). A global survey using DNA barcoding detected mislabeling of 30% of seafood samples; as such, tighter regulations have been followed in many countries (Pardo et al., 2018). DNA barcoding benefits from its integration with high-throughput sequencing techniques. The Earth BioGenome Project aims to sequence all the known eukaryotic species. Lewin et al. state that DNA barcoding plays a key role. This large database provides new views of evolution and interspecies interactions (Antil et al., 2023; A. David, J. Deepa Arul Priya, & A. Gautam, 2024).

DNA barcoding aids in agriculture by revealing complex pest-species interactions. This study identified cryptic cotton pest species (Chac & Thinh, 2023), which will facilitate the development of targeted pest strategies. In paleoecology, sediment core DNA barcoding reconstructs paleoecosystems and provides critical data to understand the long-term impacts of climate change (Gvozdenac, Dedić, Mikić, Ovuka, & Miladinović, 2022).

CRISPR-based techniques have recently offered highly specific genetic detection (Li, Wang, Xu, Wang, & Yang, 2023). Gootenberg et al. (2017) developed SHERLOCK, which detects specific genetic sequences with a single-base precision. This improves disease diagnostics and environmental monitoring (Gootenberg et al., 2017). Metabarcoding in microbial ecology has shown that soil biodiversity is several orders of magnitude higher than previously estimated, improving our understanding of ecosystem functions (Abdelfattah, Malacrinò, Wisniewski, Cacciola, & Schena, 2018; Nørgaard et al., 2021).

Table 2. A Comprehensive Overview of DNA Barcoding: Applications, Advantages, and Challenges. This table shows the diverse applications of DNA barcoding, highlighting its use in species identification, forensics, conservation, and agriculture. It addresses challenges, such as intraspecific variations, hybridization, database limitations, and ethical considerations, along with opportunities for innovation.

Application	Description	Advantages	Challenges	References
Species Identification and Delimitation	Utilized for discerning and categorizing species with unparalleled precision, particularly in ecological and evolutionary studies.	- Precision in species identification - Valuable for ecologicaland evolutionary studies	- Reliance on comprehensive reference databases - Debates on protocol standardization	(Hebert, Ratnasingham et al. 2016)

Forensic Science	This would be a strong tool in identifying biological samples for forensic purposes to aid in criminal investigations.	- This improves the toolkit available to be used within the forensic area for species identification from trace evidence but is dependent on completeness of the database.	- It does, however, raise ethical concerns in forensic applications	(Hefetz 2023)
Monitoring and Conservation of Endangered Species	It enriches conservation efforts by allowing for species detection without being invasive. demonstrated its power in the surveying of biodiversity in challenging environments.	- Noninvasive biodiversity surveying and detection of species	- Database limitationsin certain taxonomic groups - Potential misidentifications in databases	(Deiner, Bik et al. 2017, Deiner, Bik et al. 2017)
Agricultural and Food Industry Applications	The system does not only limit to the identification but can assure quality control in the agro and food industry sector.	This technology ensures proper tracking in the entire supply chain.	- Need for comprehensive reference libraries - Ethical concerns related to food sourcing	(Fanzo 2015)

Species Identification and Delimitation

Forensic Science:

DNA barcoding is an effective tool for identifying biological evidence in forensics studies. The use of DNA barcoding to clarify criminal investigations by identifying species trace evidence. Generally, the traceability of the origin of biological materials maximizes the forensic toolkit and enhances tools for use in unraveling complex cases (Elkins & Zeller, 2021; Hefetz, 2023). The development of DNA barcoding has allowed for new methods and databases to instantiate it for higher levels of accuracy and efficiency. DNA barcoding has been combined with other forensic techniques, such as traditional DNA fingerprinting and microscopy, to obtain an inclusive picture of the crime scene (Arenas et al., 2017; Shadrin, 2021).

Monitoring and conserving endangered species

In general, DNA barcoding has enriched the face of landscapes regarding major-scale conservation, especially since the implementation of species surveillance and the preservation of landscapes of concern. A good example of such research has pointed out that DNA barcoding facilitates non-invasive species detection by presenting a powerful tool for biodiversity monitoring in challenging environments. Therefore, biodiversity conservation plays a significant role in DNA barcoding, even as habitats continue to be under pressure from anthropogenic activities (Chac & Thinh, 2023).

Biodiversity is under severe pressure from anthropogenic habitat loss, pollution, and climate change, which have pushed a significant number of species to extinction. DNA barcoding is a central step in this context (Pfenning-Butterworth et al., 2024). In addition to identifying threats by detecting early signs of population decline, it also monitors the effectiveness of conservation, which enables readjustments in strategies, as needed. By prioritizing resources, DNA barcoding directs efforts toward the species and habitats that are most in need. DNA barcoding in conservation does indeed seem to be set to achieve much with improved technologies and analytical methods that increase accuracy, speed, and decreases costs (Zhu, Liu, Qiu, Dai, & Gao, 2022). Integrative approaches that couple DNA barcoding with tools such as remote sensing, citizen science, and habitat restoration will also emerge to foster comprehensive conservation programs (Sheth & Thaker, 2017).

Agricultural and Food Industry Applications

Beyond mere identification, DNA barcoding has gone to the extent of providing a linchpin for quality control and traceability in agricultural

and food sectors. Recent examples, including those of Bhattacharya et al. (Bhattacharya et al., 2016), have shown that DNA barcoding ensures the authenticity of food products with extreme accuracy across the entire supply chain, thereby protecting consumers from fraud. Such uses resonate with growing demand to ensure transparency in sourcing and safety.

DNA barcoding has radically changed the food industry by making it more open and traceable, and giving consumers the right decision-making power over their purchases. It play a critical role in enhancing food safety by preventing fraudulent activities and ensuring that products are authentic and contamination-free (Shokralla, Hellberg, Handy, King, & Hajibabaei, 2015). DNA barcoding facilitates efficient monitoring and tracking; hence, robust supply chain management provides better quality control and risk-mitigation measures (Shokralla et al., 2015). When there is growing awareness among consumers, the demand for transparency in food production and sourcing is also increasing. As food safety and traceability become priorities, the need for regulatory bodies to adopt technologies such as DNA barcoding will continue to increase (Shokralla et al., 2015). In the future, DNA barcoding will continue to be used to examine the use of food additives, and contaminant. This will be further merged with blockchain and sensor networks for end-to-end food-tracing systems. In conclusion, DNA barcoding plays a central role in the transformation of agricultural and food industries to ensure trust, safety, and accountability in food systems (Parveen et al., 2016; Shokralla et al., 2015).

Opinion and Insights:

Recognizing the advantages of DNA barcoding across a spectrum of applications enables us to move through some of the subtleties involved. Relying on DNA sequences, the availability of large reference databases is limited when such databases do not exist or are only partially available. Further debate exists regarding the standardization of protocols, and strong efforts by representatives of the scientific community are required to establish uniform practices that lead to reproducibility.

In short, the revelation of the potential for DNA-barcoding cracks opens a horizon that is replete with opportunities. Therefore, this review aims to identify recent scientific discoveries in as much detail as possible and shed light on the applications of this technique in species identification, forensic science, conservation, and the agricultural sector. As revealed in the intricate realm of DNA barcoding, a monumental tap on the back is due to acknowledgment of its shortcomings.

4. Challenges:

Although DNA barcoding is a promising tool for species identification that utilizes 600-800 base pair of cytochrome oxidase I as a mitochondrial gene to identify species (Imtiaz, Nor, & Naim, 2017). Several challenges must be addressed to ensure effective implementation. Intraspecific and intragenomic variation is considered a major challenge because variations within species and multiple copies of barcode regions within an individual's genome can complicate accurate identification (Imtiaz et al., 2017; Mishra, Sharma, Das, Pande, & Singh, 2021). Thus, hybridization and introgression complicate DNA barcoding, particularly in the case of interspecific hybrids with gene flow between taxa. Accurate interpretation requires advanced analytical tools. Notably, more advanced analytical tools are required to correct DNA barcoding results. Some studies have highlighted the need for intragenomic variation-based considerations to achieve accurate species delimitation and phylogenetic analysis (Figure 3). Although the internal transcribed spacer region of nuclear ribosomal DNA is a common marker in DNA barcoding, its utility may be influenced by intragenomic variation (Kuzmina et al., 2017; Mishra et al., 2021; Paloi, Mhuantong, Luangsa-Ard, & Kobmoo, 2021).

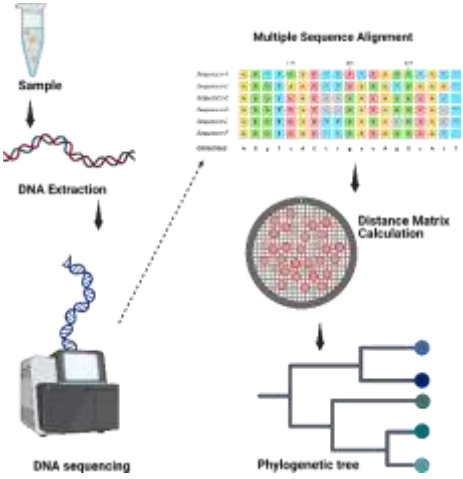

Figure 3. Phylogenetic Tree Construction from DNA Barcode Sequences.

Intraspecific and intragenomic variation

Although DNA barcoding is particularly adept at distinguishing between species, it is problematic to consider within-species and within-genome variabilities. There will certainly be variations within any species, and within an individual's genome, there may be more than one copy of a particular barcode region (Astrin et al., 2016; Raupach, Rulik, & Spelda, 2022). For example, Raupach et al. There is a delicate balance between the need for species specificity and inherent genetic diversity within populations (Raupach et al., 2022). However, according to Astrin et al., in some insect species, the intraspecific variation in the *COI* gene may be as high as 3%, which may lead to misidentification. Several researchers have suggested that this can be overcome using multiple barcoding regions (Astrin et al., 2016). For instance, Osman et al. showed improved resolution at the species level for plants when the standard markers *rbc*L and *mat*K were combined with the intergenic spacer trnH-psbA (Osman, 2024).

Hybridization and Introgression

Simple hybridization and introgression are sometimes sufficiently complex to preclude simple application of DNA barcoding. The threat of interspecific hybrids and the continuous introgression of genes between hybridizing taxa do not make a clear distinction necessary for barcoding. A recent example is the study of hybrid zones (Komarova & Lavrenchenko, 2022; Martin & Jiggins, 2017). Hence, the current challenge is not only the detection of the hybrids themselves, but also their dynamics with respect to introgression, for which advanced analytical tools and interpretations are warranted. To overcome this drawback, more advanced machine-learning algorithms can be used. Kim et al. achieved 95% accuracy in species assignment when applied to barcode data for hybridizing fish species (S. Kim, Eo, Koo, Choi, & Kim, 2010).

Database Limitations and Quality Issues

The efficiency of DNA barcoding strictly depends on the details and reliability of the reference databases. Incompleteness or bias in favor of certain taxonomic groups within a database poses a challenge. This underlines the urgency for constant maintenance and improvement of libraries (Antil et al., 2023; Fan, Hui, Yu, & Chu, 2014). An example was provided by Miller et al. The inaccuracies introduced by misidentifications and outdated taxonomies in databases are full of errors. This requires incredible vigilance and constant improvement. Analysis of the Barcode of Life Data System revealed taxonomic biases and over- or under-representation of some groups (Jin, Kim, Kim, & Park, 2020). Along this line, initiatives such as the Earth BioGenome Project have been proposed for sequencing eukaryotic biodiversity. On the other hand, it has been reported that a machine learning approach that flags barcode database misidentifications increases the data quality (Lewin et al., 2022; Lewin et al., 2018).

Ethical Considerations in DNA Barcoding

In its quest to become a mainstream tool, DNA barcoding has become increasingly entangled with a myriad of ethical issues. The misuse of genetic information raises privacy concerns, particularly in forensic applications. Jones and Salter conducted a study on ethics

related to DNA barcoding in 2018, focusing mainly on informed consent, ownership of data, and questions related to responsible use (Antil et al., 2023). For scientific development to be balanced by ethical principles, ensuring public trust and responsible deployment is imperative. The International Barcode of Life (iBOL) made institutionalized guidelines for ethical sample collection and data sharing. Further discussion is needed to address these rising DNA barcoding challenges to its ethical conduct, such as its use in environmental monitoring and possible concerns for indigenous rights (Anisha David, J. Deepa Arul Priya, & Akash Gautam, 2024; Rodger Gwiazdowski, 2024; Ma, 2015; Ratnasingham, Wei, Chan, Agda, Agda, Ballesteros-Mejia, Boutou, El Bastami, Ma, & Manjunath, 2024; Vernooy, Haribabu, Ruiz Muller, Vogel, & Hebert, 2010).

Opinion and Insights:

Challenges in DNA barcoding underscore the dynamic nature of molecular taxonomic development. Although significant, these challenges offer opportunities for innovative improvement. The future appears promising for international collaborations aimed at addressing critical shortcomings in databases aligned with emerging sequencing technologies. It is crucial to adopt a holistic approach that fully integrates molecular data with morphological and ecological information to ensure that the complexities of DNA barcoding meaningfully contribute to the biological understanding (De-Kayne et al., 2021; Joly et al., 2014; Wang et al., 2024).

Several key steps are required to overcome these challenges associated with DNA barcoding. These include addressing intraspecific and intragenomic variations to enhance resolution, developing robust analytical tools for hybridization studies, and improving database quality and coverage while adhering to ethical standards for data use and interpretation (Chac & Thinh, 2023). By addressing these challenges, DNA barcoding can continue to evolve into a valuable tool for species identification and biodiversity research. Ongoing discussions on intraspecific variations, hybridization dynamics, database limitations, and ethical considerations must further enrich scientific discourse, moving the field towards more robust and reliable applications (Mir et al., 2021; Rodriques et al., 2019).

As highlighted in the review of the potential of DNA barcoding, this dialectic between challenges and advancements propels the scientific community toward a more nuanced and comprehensive understanding of the biological world. Continuous refinement of DNA barcoding techniques and methodologies promises to unlock new insights into biodiversity and ecological relationships, ultimately contributing to more effective conservation strategies and environmental management practices (Bolotin et al., 2015; Stuart, Srivastava, Madad, Lareau, & Satija, 2021).

5. Integration with Other Technologies

Metabarcoding and Environmental DNA (eDNA):

A crucial advancement in biodiversity exploration and monitoring is the synergy between DNA barcoding, metabarcoding, and environmental DNA (eDNA) technologies (Carvalho et al., 2024). Metabarcoding, first introduced by Valentini et al. in 2006 (Valentini et al., 2016) and further developed by Taberlet et al. in 2018, has significantly expanded the scope of DNA barcoding. This technique enables parallel sequencing of numerous DNA fragments from environmental samples using current microbial tagging systems. This approach not only enhances species identification but also provides a comprehensive view of the entire ecosystem (Taberlet, Coissac, Pompanon, Brochmann, & Willerslev, 2012).

The integration of eDNA further expands research possibilities by allowing species detection without direct observation as shown in Table 2. This development has revolutionized ecological studies and environmental monitoring practices. By combining these technologies, researchers can gain deeper insights into

biodiversity and ecosystem dynamics, leading to more effective conservation strategies and environmental management

(Carvalho et al., 2024; Lefrancois, Labeille, Marquès, Robert, & Valentini, 2024; Martinelli Marín, Lasso Alcala, & Caballero, 2024).

Table 3. Summary of Metabarcoding and eDNA Analysis: Applications, Advantages, andChallenges

Aspect	Description	Applications	Advantages	Challenges	References
Definition and Overview	Metabarcoding involves high-throughput sequencing of genetic markers to identify multiple species within a sample simultaneously. Environmental DNA (eDNA) refers to genetic material extracted from environmental samples like soil or water.	- Biodiversity monitoring in ecosystems - Assessing communitystructures in aquatic environments - Studying microbialdiversity in soil - Detecting invasive species in natural habitats	- Simultaneous identification ofmultiple species - High sensitivity indetecting rare or elusive species - Non-invasive monitoring of ecosystems	- Variability in eDNApersistence and degradation rates - Potential contamination during sample collection and processing	(Hatzenbuhler, Kelly et al. 2017, Ríos-Castro, Romero et al. 2021, Jiang, Lusana et al. 2022)
Applications for Biodiversity Monitoring	Metabarcoding and eDNA are pivotal in monitoring and assessing biodiversity in various ecosystems. The techniques offer a comprehensive understanding of species composition and distribution.	- Assessing changes in species richness and abundance over time - Monitoring rare or endangered species - Identifying cryptic or hard-to-detect species	- Comprehensive insight into biodiversity dynamics - Early detection of shifts in ecosystem health - Efficient monitoring of elusive or rare species	- Standardization challenges in data analysis and interpretation	(Beng et al., 2016; Hatzenbuhler et al., 2017)
Aquatic Ecosystem Studies	Metabarcoding and eDNA are extensively employed in studying aquatic ecosystems, providing a non-invasive approach to monitoring aquatic biodiversity.	- Assessing fish and amphibian diversity in rivers and lakes - Detecting the presence of aquatic pathogens - Monitoring changes in microbial communities	- Efficient detection of fish and amphibian species without physical capture - Early detection of aquatic diseases through microbial community analysis	- Challenges in distinguishing eDNA from different organisms in complex aquatic environments	(Kelly, Port, Yamahara, & Crowder, 2014; Sahu, Kumar, Singh, & Singh, 2023)
Soil Microbial Diversity	Metabarcoding techniques are employed to study microbial diversity in soil ecosystems. eDNA analysis provides insights into the complex microbial communities present in soil.	- Understanding the role of microbes in nutrient cycling - Assessing the impact of land use on soil microbial communities - Studying the effects of climate change on soil biodiversity	- High-throughput analysis of diverse soil microbial communities - Detection of rare or novel microbial taxa in soil	- Challenges in accurately quantifying microbial abundance and diversity from eDNA samples	(Frindte, Pape, Werner, Löffler, & Knief, 2019; Lombard, Prestat, van Elsas, & Simonet, 2011)
Detection of Invasive Species	Metabarcoding and eDNA analysis are crucial in the early detection and monitoring of invasive species in natural habitats.	- Identifying invasive plant and animal species in terrestrial and aquatic environments - Monitoring the spread of invasive species in ecosystems	- Early detection of invasive species before significant ecological impact - Monitoring the effectiveness of invasive species management strategies	- Challenges in distinguishing eDNA from native and invasive species in diverse ecosystems	(Evangelista , Stohlgren, Morisette, & Kumar, 2009; Piper, Cunningham , Cogan, & Blacket, 2022)

Advancements in Technology	Continuous technological advancements contribute to the effectiveness of metabarcoding and eDNA analysis.	- Implementation of high-throughput sequencing platforms - Development of bioinformatics tools for data analysis - Integration of eDNA metabarcoding with other omics approaches	- Increased speed and cost-effectiveness of DNA sequencing - Enhanced accuracy and resolution in species identification	- Rapid changes in technology may lead to challenges in standardization and compatibility	(Dully et al., 2021; Nørgaard et al., 2021)
Data Standardization and Integration	Standardization of data analysis methods and integration with other ecological data enhance the reliability and applicability of metabarcoding and eDNA studies.	- Developing standardized protocols for eDNA sample collection and processing - Integrating eDNA data with environmental metadata for comprehensive ecological assessments	- Facilitates cross-study comparisons and meta-analyses - Enhances the reproducibility and reliability of results	- Challenges in achieving global standardization due to varied environmental conditions	(Pascher, Švara, & Jungmeier, 2022; Perry et al., 2024)

Integration with Geographic Information Systems (GIS):

The incorporation of DNA barcoding into Geographic Information Systems (GIS) represents one of the latest frontiers in spatial analysis for biodiversity research (Afifi, Azab, Ali, Ghazy, & El-Tabakh, 2024; Čandek & Kuntner, 2015; Moser et al., 2014). Overlaying genetic data with geographical information provides a more comprehensive and sensitive description of species distributions and migration patterns. This integration has proven to be effective in nature, allowing for improved predictive modelling and conservation planning. Consequently, ecological studies and conservation measures benefit from the integration of spatial and genetic data to derive more accurate and informed decisions (Bruni et al., 2015; Conflitti, Pruess, Cywinska, Powers, & Currie, 2013; Kartavtsev, 2018; Marco-Herrero, Cuesta, & González-Gordillo, 2021).

Synergy with Traditional Taxonomic Approaches

It would be counterproductive if DNA barcoding and traditional methodologies did not complement each other to maximize their joint potential for exploring biodiversity (Ellis et al., 2020). An integrative taxonomic approach combining molecular data with morphological and ecological characteristics has been proposed. This integrative method not only addresses the limitations of DNA barcoding alone but also provides a more accurate representation of species diversity. The combined use of these techniques is particularly important when genetic characteristics alone are insufficient, highlighting the need for a comprehensive taxonomic approach (Mamat, Abu, & Yusoff, 2021; Song et al., 2020; B. Yang et al., 2022).

Opinion and Insights:

The integration of DNA barcoding with other modern technologies has changed the way biodiversity is researched. Metabarcoding and eDNA techniques have expanded our understanding of individual specimens to examine the entire ecosystem. Matching DNA barcode data with GIS improves spatial understanding of biodiversity, which is crucial for developing effective conservation strategies. Collaboration with traditional taxonomic approaches provided a more balanced approach.

The value of DNA barcoding stems from its integration into other technologies. This creates a comprehensive view of biological systems. As these methodologies develop, they reveal further aspects of biodiversity, highlighting the dynamic and connected nature of the biological world.

6. Future Directions: Improvements in Sequencing Technologies

DNA barcoding benefits from progress in sequencing technologies. Next-generation sequencing and the emerging third-generation methods are more efficient and accurate. Kumar et al. used Oxford Nanopore MinION platform to generate long reads (>100 kb) with 99.9% accuracy, which could resolve issues of intraspecific variation in barcoding (Kumar, Cowley, & Davis, 2019; Kumar, Davis, et al., 2019; Kumar et al., 2016). These improvements lead to faster, more accurate, and lower-cost sequencing, expanding the scope of molecular taxonomy

Exploration of Novel Marker Genes

The ideal barcode regions remain elusive, and studies have explored alternatives to the standard *COI* gene. Several studies have tested the ITS2 region as a plant barcode and found that it offers superior resolution for some groups compared to standard markers (Alsos et al., 2020; Fazekas, Kuzmina, Newmaster, & Hollingsworth, 2012; Jones et al., 2021). In recent years, multigene approaches have become increasingly popular. Liu et al. developed a three-gene barcode for land plants, using *rbc*L, *mat*K, and ITS, achieving 95% success in species identification across diverse taxa (J. Z. Liu, Erlich, & Pickrell, 2017).

Integration of Machine Learning in Data Analysis

Additionally, machine learning algorithms can be used to analyze complex DNA barcoding datasets. convolutional neural networks for species classification based on *COI* sequences from fish samples, achieving a classification accuracy of 99.5% across more than 7,000 species (Yu et al., 2021). These approaches make barcoding more precise and help interpret genetic information. Furthermore, a deep learning model identifies species based on metabarcoding data, which is much faster than the conventional methods (Sohsah, Ibrahimzada, Ayaz, & Cakmak, 2020; Yang, Wu, Chuang, & Chang, 2022).

Global Collaboration and Standardization

International cooperation can also improve DNA barcoding. For example, the International Barcode of Life (iBOL) consortium started in 2010. Their BIOSCAN project, launched in 2019, aims to barcode 2 million species by 2026 (Hobern, 2021). These initiatives aim to address challenges in database quality, marker selection, and methodological standardization.

Another key collaborative effort was the Earth BioGenome Project announced in 2018. This project aims to sequence and catalog all known eukaryotic species within ten years, which will provide invaluable data for barcoding efforts (Lewin et al., 2022; Lewin et al., 2018).

We believe that the future of DNA barcoding will depend on combining technological, methodological, and global collaborative processes. Second-generation sequencing technologies and novel marker genes can improve the accuracy and speed of species identification, whereas the integration of machine learning may significantly improve data analysis. International initiatives can promote standardization and global biodiversity coverage.

However, these challenges will persist in the future. Ethical issues related to data ownership and access should also be considered. It has been reported that global barcoding projects must address questions of equitable benefit-sharing for genetic resources from regions rich in biodiversity, but poor in economic capacity ("A DNA barcode for land plants," 2009; Seberg & Petersen, 2009).

DNA barcoding is becoming an increasingly critical component of biodiversity research, conservation, and

understanding the genetic diversity of life. Success in this field requires balanced technological progress with ethical considerations and a globally collaborative approach to understanding the planet's biodiversity (A. David et al., 2024; I. S. Kim, 2023; Shumskaya, 2024).

7. Conclusions

DNA barcoding presents substantial opportunities. It has several applications, challenges, and possibilities for its integration. Researchers have used this method for species identification, conservation, forensic science, and agriculture. Its integration includes metabarcoding, GIS, and traditional taxonomies. This broadens the scope and adds depth to biodiversity studies. DNA barcoding advances molecular taxonomy by revealing the nature of the variation in species. It faces challenges such as hybridization, database limitations, and ethical concerns. These issues present new opportunities for innovation; combining DNA barcoding with advances in sequencing, the identification of novel marker genes, and machine learning can improve accuracy and efficiency. Continued research on DNA barcoding is therefore crucial. The global scientific community should address the challenges in DNA barcoding, including ethics and the expansion of reference databases. Advancing this field requires collaboration across both disciplinary and geographical boundaries. DNA barcoding has the potential to unlock the mysteries of biodiversity. This field calls for molecular taxonomy to bridge gaps in scientific knowledge.

Authors contribution: A.A. and A.A.A.A. conceptualized the study. A.A., A.R., M.B.A, O.G., E.Q., A.A., and A.A.A.A. contributed equally to the writing of the original draft. All authors participated in reviewing and editing the manuscript, and all agreed on the final version of the manuscript.

Data availability: No new data were generated from this Manuscript.

Conflict of interest: Authors declare no conflict of interest.

Funding: The manuscript receives no fundi

WORKS CITED

Abdelfattah, A., Malacrinò, A., Wisniewski, M., Cacciola, S. O., & Schena, L. (2018). Metabarcoding: A powerful tool to investigate microbial communities and shape future plant protection strategies. *Biological Control, 120*, 1-10. doi:https://doi.org/10.1016/j.biocontrol.2017.07.009

Afifi, M. A. M., Azab, A. M., Ali, E., Ghazy, A., & El-Tabakh, M. A. M. (2024). DNA barcoding, phylogeography and evolutionary dynamics of Chrysichthys auratus. *Gene, 917*, 148448. doi:10.1016/j.gene.2024.148448

Alsos, I. G., Lavergne, S., Merkel, M. K. F., Boleda, M., Lammers, Y., Alberti, A., . . . Coissac, E. (2020). The Treasure Vault Can be Opened: Large-Scale Genome Skimming Works Well Using Herbarium and Silica Gel Dried Material. *Plants (Basel), 9*(4). doi:10.3390/plants9040432

Anslan, S., Mikryukov, V., Armolaitis, K., Ankuda, J., Lazdina, D., Makovskis, K., . . . Tedersoo, L. (2021). Highly comparable metabarcoding results from MGI-Tech and Illumina sequencing platforms. *PeerJ, 9*, e12254.

Antil, S., Abraham, J. S., Sripoorna, S., Maurya, S., Dagar, J., Makhija, S., . . . Toteja, R. (2023). DNA barcoding, an effective tool for species identification: a review. *Molecular Biology Reports, 50*(1), 761-775. doi:10.1007/s11033-022-08015-7

Arenas, M., Pereira, F., Oliveira, M., Pinto, N., Lopes, A. M., Gomes, V., . . . Amorim, A. (2017). Forensic genetics and genomics: Much more than just a human affair. *PLoS genetics, 13*(9), e1006960.

Astrin, J. J., Höfer, H., Spelda, J., Holstein, J., Bayer, S., Hendrich, L., . . . Lemke, M. (2016). Towards a DNA barcode reference database for spiders and harvestmen of Germany. *PloS one, 11*(9), e0162624.

Bammer, G., O'Rourke, M., O'Connell, D., Neuhauser, L., Midgley, G., Klein, J. T., . . . Richardson, G. P.

(2020). Expertise in research integration and implementation for tackling complex problems: when is it needed, where can it be found and how can it be strengthened? *Palgrave Communications, 6*(1), 5. doi:10.1057/s41599-019-0380-0

Belwal, S., & Jadeja, S. (2024). Biomarkers for the biodiversity loss and restoration of degraded ecosystems. In *Biomarkers in Environmental and Human Health Biomonitoring* (pp. 227-246): Elsevier.

Beng, C., Tomlinson, K. W., Shen XianHui, S. X., Surget-Groba, Y., Hughes, A. C., Corlett, R. T., & Slik, J. W. F. (2016). The utility of DNA metabarcoding for studying the response of arthropod diversity and composition to land-use change in the tropics.

Bezeng, B. S., Davies, T. J., Daru, B. H., Kabongo, R. M., Maurin, O., Yessoufou, K., . . . Van der Bank, M. (2017). Ten years of barcoding at the African Centre for DNA Barcoding. *Genome, 60*(7), 629-638.

Bhattacharya, M., Sharma, A. R., Patra, B. C., Sharma, G., Seo, E.-M., Nam, J.-S., . . . Lee, S.-S. (2016). DNA barcoding to fishes: current status and future directions. *Mitochondrial DNA Part A, 27*(4), 2744-2752.

Bohmann, K., Elbrecht, V., Carøe, C., Bista, I., Leese, F., Bunce, M., . . . Creer, S. (2022). Strategies for sample labelling and library preparation in DNA metabarcoding studies. *Molecular ecology resources, 22*(4), 1231-1246.

Bolotin, D. A., Poslavsky, S., Mitrophanov, I., Shugay, M., Mamedov, I. Z., Putintseva, E. V., & Chudakov, D. M. (2015). MiXCR: software for comprehensive adaptive immunity profiling. *Nature methods, 12*(5), 380-381.

Braukmann, T. W. A., Ivanova, N. V., Prosser, S. W. J., Elbrecht, V., Steinke, D., Ratnasingham, S., . . . Hebert, P. D. N. (2019). Metabarcoding a diverse arthropod mock community. *Molecular ecology resources, 19*(3), 711-727.

Bruni, I., Galimberti, A., Caridi, L., Scaccabarozzi, D., De Mattia, F., Casiraghi, M., & Labra, M. (2015). A DNA barcoding approach to identify plant species in multiflower honey. *Food Chem, 170*, 308-315. doi:10.1016/j.foodchem.2014.08.060

Čandek, K., & Kuntner, M. (2015). DNA barcoding gap: reliable species identification over morphological and geographical scales. *Mol Ecol Resour, 15*(2), 268-277. doi:10.1111/1755-0998.12304

Carvalho, C. O., Gromstad, W., Dunthorn, M., Karlsen, H. E., Schrøder-Nielsen, A., Ready, J. S., . . . Mauvisseau, Q. (2024). Harnessing eDNA metabarcoding to investigate fish community composition and its seasonal changes in the Oslo fjord. *Scientific reports, 14*(1), 10154.

Chac, L. D., & Thinh, B. B. (2023). Species Identification through DNA Barcoding and Its Applications: A Review. *Biology Bulletin, 50*(6), 1143-1156. doi:10.1134/S106235902360229X

Chalermwong, P., Panthum, T., Wattanadilokcahtkun, P., Ariyaraphong, N., Thong, T., Srikampa, P., . . . Srikulnath, K. (2023). Overcoming taxonomic challenges in DNA barcoding for improvement of identification and preservation of clariid catfish species. *Genomics Inform, 21*(3), e39. doi:10.5808/gi.23038

Che, J., Chen, H. M., Yang, J. X., Jin, J. Q., Jiang, K. E., Yuan, Z. Y., . . . Zhang, Y. P. (2012). Universal COI primers for DNA barcoding amphibians. *Molecular ecology resources, 12*(2), 247-258.

China Plant, B. O. L. G., Li, D.-Z., Gao, L.-M., Li, H.-T., Wang, H., Ge, X.-J., . . . Chen, S.-L. (2011). Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences, 108*(49), 19641-19646.

Conflitti, I. M., Pruess, K. P., Cywinska, A., Powers, T. O., & Currie, D. C. (2013). DNA barcoding distinguishes pest species of the black fly genus Cnephia (Diptera: Simuliidae). *J Med Entomol, 50*(6), 1250-1260. doi:10.1603/me13063

Dallas, H. F. (2021). Rapid Bioassessment Protocols Using Aquatic Macroinvertebrates in Africa-Considerations for Regional Adaptation of Existing Biotic Indices. *Frontiers in Water, 3*. doi:10.3389/frwa.2021.628227

David, A., Deepa Arul Priya, J., & Gautam, A. (2024). DNA Sequencing Technologies and DNA Barcoding.

Methods Mol Biol, 2744, 139-154. doi:10.1007/978-1-0716-3581-0_8

David, A., Deepa Arul Priya, J., & Gautam, A. (2024). DNA Sequencing Technologies and DNA Barcoding. In *DNA Barcoding: Methods and Protocols* (pp. 139-154): Springer.

De-Kayne, R., Frei, D., Greenway, R., Mendes, S. L., Retel, C., & Feulner, P. G. D. (2021). Sequencing platform shifts provide opportunities but pose challenges for combining genomic data sets. *Mol Ecol Resour, 21*(3), 653-660. doi:10.1111/1755-0998.13309

de Medeiros, B. A. S., Cai, L., Flynn, P. J., Yan, Y., Duan, X., Marinho, L. C., . . . Davis, C. (2024). A universal DNA barcode for the Tree of Life.

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., . . . Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Mol Ecol, 26*(21), 5872-5895. doi:10.1111/mec.14350

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., . . . De Vere, N. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular ecology, 26*(21), 5872-5895.

DeSalle, R., & Goldstein, P. (2019). Review and Interpretation of Trends in DNA Barcoding. *Frontiers in Ecology and Evolution, 7*. doi:10.3389/fevo.2019.00302

A DNA barcode for land plants. (2009). *Proc Natl Acad Sci U S A, 106*(31), 12794-12797. doi:10.1073/pnas.0905845106

Duan, H., Wang, W., Zeng, Y., Guo, M., & Zhou, Y. (2019). The screening and identification of DNA barcode sequences for Rehmannia. *Scientific reports, 9*(1), 17295.

Dully, V., Balliet, H., Frühe, L., Däumer, M., Thielen, A., Gallie, S., . . . Stoeck, T. (2021). Robustness, sensitivity and reproducibility of eDNA metabarcoding as an environmental biomonitoring tool in coastal salmon aquaculture–An inter-laboratory study. *Ecological Indicators, 121*, 107049.

Elkins, K. M., & Zeller, C. B. (2021). *Next generation sequencing in forensic science: a primer*: CRC Press.

Ellis, J. L., Jacobs, M., Dijkstra, J., van Laar, H., Cant, J. P., Tulpan, D., & Ferguson, N. (2020). Review: Synergy between mechanistic modelling and data-driven models for modern animal production systems in the era of big data. *Animal, 14*(S2), s223-s237. doi:10.1017/s1751731120000312

Evangelista, P. H., Stohlgren, T. J., Morisette, J. T., & Kumar, S. (2009). Mapping invasive tamarisk (Tamarix): a comparison of single-scene and time-series analyses of remotely sensed data. *Remote Sensing, 1*(3), 519-533.

Fan, L., Hui, J. H. L., Yu, Z. G., & Chu, K. H. (2014). arcoding: composition vector-based software for rapid species identification based on DNA barcoding. *Molecular ecology resources, 14*(4), 871-881. doi:https://doi.org/10.1111/1755-0998.12235

Fanzo, J. (2015). Ethical issues for human nutrition in the context of global food security and sustainable development. *Global Food Security, 7*, 15-23. doi:https://doi.org/10.1016/j.gfs.2015.11.001

Fasullo, M., & Dolan, M. (2022). The continuing evolution of barcode applications: Functional toxicology to cell lineage. *Experimental Biology and Medicine, 247*(23), 2119-2127.

Fazekas, A. J., Kesanakurti, P. R., Burgess, K. S., Percy, D. M., Graham, S. W., Barrett, S. C., . . . Husband, B. C. (2009). Are plant species inherently harder to discriminate than animal species using DNA barcoding markers? *Mol Ecol Resour, 9 Suppl s1*, 130-139. doi:10.1111/j.1755-0998.2009.02652.x

Fazekas, A. J., Kuzmina, M. L., Newmaster, S. G., & Hollingsworth, P. M. (2012). DNA barcoding methods for land plants. *Methods Mol Biol, 858*, 223-252. doi:10.1007/978-1-61779-591-6_11

Frindte, K., Pape, R., Werner, K., Löffler, J., & Knief, C. (2019). Temperature and soil moisture control microbial community composition in an arctic–alpine ecosystem along elevational and micro-topographic gradients. *The ISME Journal, 13*(8), 2031-2043.

Gao, Z., Liu, Y., Wang, X., Wei, X., & Han, J. (2019). DNA mini-barcoding: a derived barcoding method for herbal molecular identification. *Frontiers in plant science, 10*, 987.

Ghosh, S., Bankura, B., & Das, M. (2016). DNA Barcoding: a tool to assess and conserve Marine Biodiversity.

DNA Barcoding in Marine Perspectives: Assessment and Conservation of Biodiversity, 43-61.

Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., . . . Zhang, F. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science, 356*(6336), 438-442. doi:10.1126/science.aam9321

Gostel, M. R., & Kress, W. J. (2022). The expanding role of DNA barcodes: Indispensable tools for ecology, evolution, and conservation. *Diversity, 14*(3), 213.

Group, C. P. W., Hollingsworth, P. M., Forrest, L. L., Spouge, J. L., Hajibabaei, M., Ratnasingham, S., . . . Erickson, D. L. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences, 106*(31), 12794-12797.

Guo, M., Yuan, C., Tao, L., Cai, Y., & Zhang, W. (2022). Life barcoded by DNA barcodes. *Conservation Genetics Resources, 14*(4), 351-365. doi:10.1007/s12686-022-01291-2

Gvozdenac, S., Dedić, B., Mikić, S., Ovuka, J., & Miladinović, D. (2022). Impact of Climate Change on Integrated Pest Management Strategies. In *Climate Change and Agriculture* (pp. 311-372).

Gwiazdowski, R. (2024). Principles for Constructing DNA Barcode Reference Libraries. *Methods Mol Biol, 2744*, 491-502. doi:10.1007/978-1-0716-3581-0_29

Gwiazdowski, R. (2024). Principles for Constructing DNA Barcode Reference Libraries. In *DNA Barcoding: Methods and Protocols* (pp. 491-502): Springer.

Hakimzadeh, A., Abdala Asbun, A., Albanese, D., Bernard, M., Buchner, D., Callahan, B., . . . Brandström Durling, M. (2024). A pile of pipelines: An overview of the bioinformatics software for metabarcoding data analyses. *Molecular ecology resources, 24*(5), e13847.

Hatzenbuhler, C., Kelly, J. R., Martinson, J., Okum, S., & Pilgrim, E. (2017). Sensitivity and accuracy of high-throughput metabarcoding methods for early detection of invasive fish species. *Scientific reports, 7*(1), 46393.

Hebert, P. D., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proc Biol Sci, 270*(1512), 313-321. doi:10.1098/rspb.2002.2218

Hebert, P. D., Ratnasingham, S., Zakharov, E. V., Telfer, A. C., Levesque-Beaudin, V., Milton, M. A., . . . deWaard, J. R. (2016). Counting animal species with DNA barcodes: Canadian insects. *Philos Trans R Soc Lond B Biol Sci, 371*(1702). doi:10.1098/rstb.2015.0333

Hebert, P. D. N., Braukmann, T. W. A., Prosser, S. W. J., Ratnasingham, S., DeWaard, J. R., Ivanova, N. V., . . . Sones, J. E. (2018). A Sequel to Sanger: amplicon sequencing that scales. *BMC genomics, 19*, 1-14.

Hebert, P. D. N., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1512), 313-321.

Hefetz, I. (2023). Mapping AI-ethics' dilemmas in forensic case work: To trust AI or not? *Forensic Science International, 350*, 111807. doi:https://doi.org/10.1016/j.forsciint.2023.111807

Hobern, D. (2021). BIOSCAN: DNA barcoding to accelerate taxonomy and biogeography for conservation and sustainability. *Genome, 64*(3), 161-164.

Hollingsworth, P. M., Graham, S. W., & Little, D. P. (2011). Choosing and using a plant DNA barcode. *PloS one, 6*(5), e19254.

Imtiaz, A., Nor, S. A. M., & Naim, D. M. (2017). Progress and potential of DNA barcoding for species identification of fish species. *Biodiversitas Journal of Biological Diversity, 18*(4), 1394-1405.

Ivanova, N. V., Dewaard, J. R., & Hebert, P. D. N. (2006). An inexpensive, automation-friendly protocol for recovering high-quality DNA. *Molecular ecology notes, 6*(4), 998-1002.

Jalali, S. K., Ojha, R., & Venkatesan, T. (2015). DNA barcoding for identification of agriculturally important insects. *New horizons in insect science: Towards sustainable pest management*, 13-23.

Jiang, R., Lusana, J. L., & Chen, Y. (2022). High-throughput DNA metabarcoding as an approach for ichthyoplankton survey in Oujiang River Estuary, China. *Diversity, 14*(12), 1111.

Jin, S., Kim, K. Y., Kim, M.-S., & Park, C. (2020). An assessment of the taxonomic reliability of DNA barcode sequences in publicly available databases. *Algae, 35*(3), 293-301.

Joly, S., Davies, T. J., Archambault, A., Bruneau, A., Derry, A., Kembel, S. W., . . . Wheeler, T. A. (2014). Ecology in the age of DNA barcoding: the resource, the promise and the challenges ahead. *Molecular ecology resources, 14*(2), 221-232.

Jones, L., Twyford, A. D., Ford, C. R., Rich, T. C. G., Davies, H., Forrest, L. L., . . . de Vere, N. (2021). Barcode UK: A complete DNA barcoding resource for the flowering plants and conifers of the United Kingdom. *Mol Ecol Resour, 21*(6), 2050-2062. doi:10.1111/1755-0998.13388

Kang, Y., Deng, Z., Zang, R., & Long, W. (2017). DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests. *Scientific reports, 7*(1), 12564.

Kartavtsev, Y. P. (2018). Barcode index number, taxonomic rank and modes of speciation: examples from fish. *Mitochondrial DNA A DNA Mapp Seq Anal, 29*(4), 535-542. doi:10.1080/24701394.2017.1315570

Keck, F., Blackman, R. C., Bossart, R., Brantschen, J., Couton, M., Hürlemann, S., . . . Altermatt, F. (2022). Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. *Molecular ecology, 31*(6), 1820-1835.

Kelly, R. P., Port, J. A., Yamahara, K. M., & Crowder, L. B. (2014). Using environmental DNA to census marine fishes in a large mesocosm. *PloS one, 9*(1), e86175.

Kim, I. S. (2023). DNA Barcoding Technology for Lineage Recording and Tracing to Resolve Cell Fate Determination. *Cells, 13*(1). doi:10.3390/cells13010027

Kim, S., Eo, H.-S., Koo, H., Choi, J.-K., & Kim, W. (2010). DNA barcode-based molecular identification system for fish species. *Molecules and cells, 30*, 507-512.

Komarova, V. A., & Lavrenchenko, L. A. (2022). Approaches to the Detection of Hybridization Events and Genetic Introgression upon Phylogenetic Incongruence. *Biology Bulletin Reviews, 12*(3), 240-253. doi:10.1134/S2079086422030045

Kress, W. J. (2017). Plant DNA barcodes: Applications today and in the future. *Journal of Systematics and Evolution, 55*(4), 291-307. doi:https://doi.org/10.1111/jse.12254

Kress, W. J., & Erickson, D. L. (2008). DNA barcodes: genes, genomics, and bioinformatics. *Proceedings of the National Academy of Sciences, 105*(8), 2761-2762.

Kress, W. J., García-Robledo, C., Uriarte, M., & Erickson, D. L. (2015). DNA barcodes for ecology, evolution, and conservation. *Trends in ecology & evolution, 30*(1), 25-35. doi:https://doi.org/10.1016/j.tree.2014.10.008

Kumar, K. R., Cowley, M. J., & Davis, R. L. (2019). Next-Generation Sequencing and Emerging Technologies. *Semin Thromb Hemost, 45*(7), 661-673. doi:10.1055/s-0039-1688446

Kumar, K. R., Davis, R. L., Tchan, M. C., Wali, G. M., Mahant, N., Ng, K., . . . Sue, C. M. (2019). Whole genome sequencing for the genetic diagnosis of heterogenous dystonia phenotypes. *Parkinsonism Relat Disord, 69*, 111-118. doi:10.1016/j.parkreldis.2019.11.004

Kumar, K. R., Wali, G. M., Kamate, M., Wali, G., Minoche, A. E., Puttick, C., . . . Cowley, M. J. (2016). Defining the genetic basis of early onset hereditary spastic paraplegia using whole genome sequencing. *Neurogenetics, 17*(4), 265-270. doi:10.1007/s10048-016-0495-z

Kuzmina, M. L., Braukmann, T. W. A., Fazekas, A. J., Graham, S. W., Dewaard, S. L., Rodrigues, A., . . . Catling, P. M. (2017). Using herbarium-derived DNAs to assemble a large-scale DNA barcode library for the vascular plants of Canada. *Applications in Plant Sciences, 5*(12), 1700079.

Lahaye, R., Van der Bank, M., Bogarin, D., Warner, J., Pupulin, F., Gigot, G., . . . Savolainen, V. (2008). DNA barcoding the floras of biodiversity hotspots. *Proceedings of the National Academy of Sciences, 105*(8), 2923-2928.

Lefrancois, E., Labeille, M., Marquès, J., Robert, M., & Valentini, A. (2024). Validation of an eDNA-based method for surveying fish and crustacean communities in the rivers of the French West Indies. *Hydrobiologia*, 1-21.

Lewin, H. A., Richards, S., Lieberman Aiden, E., Allende, M. L., Archibald, J. M., Bálint, M., . . . Bertorelle, G. (2022). The earth BioGenome project 2020: Starting the clock. In (Vol. 119, pp. e2115635118): National Acad Sciences.

Lewin, H. A., Robinson, G. E., Kress, W. J., Baker, W. J., Coddington, J., Crandall, K. A., . . . Gilbert, M. T. P. (2018). Earth BioGenome Project: Sequencing life for the future of life. *Proceedings of the National Academy of Sciences, 115*(17), 4325-4333.

Li, Z.-H., Wang, J., Xu, J.-P., Wang, J., & Yang, X. (2023). Recent advances in CRISPR-based genome editing technology and its applications in cardiovascular research. *Military Medical Research, 10*(1), 12. doi:10.1186/s40779-023-00447-x

Lim, N. K. M., Tay, Y. C., Srivathsan, A., Tan, J. W. T., Kwik, J. T. B., Baloğlu, B., . . . Yeo, D. C. J. (2016). Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. *Royal Society open science, 3*(11), 160635.

Liu, J., Jiang, J., Song, S., Tornabene, L., Chabarria, R., Naylor, G. J. P., & Li, C. (2017). Multilocus DNA barcoding–species identification with multilocus data. *Scientific reports, 7*(1), 16601.

Liu, J. Z., Erlich, Y., & Pickrell, J. K. (2017). Case-control association mapping by proxy using family history of disease. *Nat Genet, 49*(3), 325-331. doi:10.1038/ng.3766

Liu, Y., Zhang, M., Chen, X., Chen, X., Hu, Y., Gao, J., . . . Du, Y. (2021). Developing an efficient DNA barcoding system to differentiate between Lilium species. *BMC plant biology, 21*, 1-13.

Lombard, N., Prestat, E., van Elsas, J. D., & Simonet, P. (2011). Soil-specific limitations for access and analysis of soil microbial communities by metagenomics. *FEMS microbiology ecology, 78*(1), 31-49.

Lyons, E., Sheridan, P., Tremmel, G., Miyano, S., & Sugano, S. (2017). Large-scale DNA barcode library generation for biomolecule identification in high-throughput screens. *Scientific reports, 7*(1), 13899.

Ma, K. (2015). DNA barcode: From species to biome. *Biodiversity Science, 23*(3), 279.

Mahmoud, A. G. Y., & Zaher, E. H. F. (2015). Why nuclear ribosomal internal transcribed spacer (ITS) has been selected as the DNA barcode for fungi. *Adv Genet Eng, 4*(119), 2169-0111.

Mamat, N., Abu, A., & Yusoff, N. R. (2021). Classification and Morphology of Rhinocypha spp. (Odonata): A Comprehensive Taxonomic Study Within the Females. *Zool Stud, 60*, e47. doi:10.6620/zs.2021.60-47

Marco-Herrero, E., Cuesta, J. A., & González-Gordillo, J. I. (2021). DNA barcoding allows identification of undescribed crab megalopas from the open sea. *Sci Rep, 11*(1), 20573. doi:10.1038/s41598-021-99486-4

Marshall, E. (2005). Taxonomy. Will DNA bar codes breathe life into classification? *Science, 307*(5712), 1037. doi:10.1126/science.307.5712.1037

Martin, S. H., & Jiggins, C. D. (2017). Interpreting the genomic landscape of introgression. *Current Opinion in Genetics & Development, 47*, 69-74. doi:https://doi.org/10.1016/j.gde.2017.08.007

Martinelli Marín, D., Lasso Alcala, C. A., & Caballero, S. (2024). eDNA metabarcoding: an effective tool for vertebrate diversity studies in the Colombian Amazon and Orinoco basins. *Frontiers in Ecology and Evolution, 12*, 1409296.

McDonald, R., Bateman, P. W., Cooper, C., van der Heyde, M., Mousavi-Derazmahalleh, M., Hedges, B. A., . . . Nevill, P. (2023). Detection of vertebrates from natural and artificial inland water bodies in a semi-arid habitat using eDNA from filtered, swept, and sediment samples. *Ecology and Evolution, 13*(4), e10014.

Mir, R. A., Bhat, K. A., Rashid, G., Ebinezer, L. B., Masi, A., Rakwal, R., . . . Zargar, S. M. (2021). DNA barcoding: a way forward to obtain deep insights about the realistic diversity of living organisms. *The Nucleus, 64*(2), 157-165. doi:10.1007/s13237-020-00330-3

Miralles, A., Puillandre, N., & Vences, M. (2024). DNA Barcoding in Species Delimitation: From Genetic Distances to Integrative Taxonomy. *Methods Mol Biol, 2744*, 77-104. doi:10.1007/978-1-0716-3581-0_4

Miralles, A., Puillandre, N., & Vences, M. (2024). DNA barcoding in species delimitation: from genetic distances to integrative taxonomy. In *DNA Barcoding: Methods and Protocols* (pp. 77-104): Springer.

Mishra, S., Sharma, G., Das, M. K., Pande, V., & Singh, O. P. (2021). Intragenomic sequence variations in the second internal transcribed spacer (ITS2) ribosomal DNA of the malaria vector Anopheles stephensi. *PloS one, 16*(6), e0253173.

Moser, W., Greter, H., Schindler, C., Allan, F., Ngandolo, B. N., Moto, D. D., . . . Zinsstag, J. (2014). The spatial and seasonal distribution of Bulinus truncatus, Bulinus forskalii and Biomphalaria pfeifferi, the intermediate host snails of schistosomiasis, in N'Djamena, Chad. *Geospat Health, 9*(1), 109-118. doi:10.4081/gh.2014.9

Nath, S., VanSlambrouck, J. T., Yao, J. W., Gullapalli, A., Razi, F., & Lu, Y. (2024). DNA barcoding of terrestrial invasive plant species in Southwest Michigan. *Plant Direct, 8*(6), e615. doi:10.1002/pld3.615

Newmaster, S. G., Grguric, M., Shanmughanandhan, D., Ramalingam, S., & Ragupathy, S. (2013). DNA barcoding detects contamination and substitution in North American herbal products. *BMC Med, 11*, 222. doi:10.1186/1741-7015-11-222

Nørgaard, L., Olesen, C. R., Trøjelsgaard, K., Pertoldi, C., Nielsen, J. L., Taberlet, P., . . . Iacolina, L. (2021). eDNA metabarcoding for biodiversity assessment, generalist predators as sampling assistants. *Scientific reports, 11*(1), 6820.

Onoh, U. C., Ogunade, J., Owoeye, E., Awakessien, S., & Asomah, J. K. (2024). Impact of Climate Change on Biodiversity and Ecosystems Services. *International Journal of Geography and Environmental Management (IJGEM), 10*(1), 77-93.

Osman, S. A. (2024). The Power of DNA Barcoding for Plant Identification. *Egyptian Journal of Chemistry, 67*(1), 633-646.

Packer, L., Gibbs, J., Sheffield, C., & Hanner, R. (2009). DNA barcoding and the mediocrity of morphology. *Molecular ecology resources, 9*, 42-50.

Paloi, S., Mhuantong, W., Luangsa-Ard, J. J., & Kobmoo, N. (2021). Using high-throughput amplicon sequencing to evaluate intragenomic variation and accuracy in species identification of Cordyceps species. *Journal of Fungi, 7*(9), 767.

Pardo, M. Á., Jiménez, E., Viðarsson, J. R., Ólafsson, K., Ólafsdóttir, G., Daníelsdóttir, A. K., & Pérez-Villareal, B. (2018). DNA barcoding revealing mislabeling of seafood in European mass caterings. *Food Control, 92*, 7-16. doi:https://doi.org/10.1016/j.foodcont.2018.04.044

Parveen, I., Gafner, S., Techen, N., Murch, S. J., & Khan, I. A. (2016). DNA barcoding for the identification of botanicals in herbal medicine and dietary supplements: strengths and limitations. *Planta Medica, 82*(14), 1225-1235.

Pascher, K., Švara, V., & Jungmeier, M. (2022). Environmental DNA-Based Methods in Biodiversity Monitoring of Protected Areas: Application Range, Limitations, and Needs. *Diversity, 14*(6). doi:10.3390/d14060463

Pentinsaari, M., Ratnasingham, S., Miller, S. E., & Hebert, P. D. N. (2020). BOLD and GenBank revisited–Do identification errors arise in the lab or in the sequence libraries? *PloS one, 15*(4), e0231814.

Perry, W. B., Seymour, M., Orsini, L., Jâms, I. B., Milner, N., Edwards, F., . . . Creer, S. (2024). An integrated spatio-temporal view of riverine biodiversity using environmental DNA metabarcoding. *Nature Communications, 15*(1), 4372. doi:10.1038/s41467-024-48640-3

Pfenning-Butterworth, A., Buckley, L. B., Drake, J. M., Farner, J. E., Farrell, M. J., Gehman, A.-L. M., . . . Davies, T. J. (2024). Interconnecting global threats: climate change, biodiversity loss, and infectious diseases. *The Lancet Planetary Health, 8*(4), e270-e283. doi:https://doi.org/10.1016/S2542-5196(24)00021-4

Piper, A. M., Cunningham, J. P., Cogan, N. O. I., & Blacket, M. J. (2022). DNA metabarcoding enables high-throughput detection of spotted wing drosophila (Drosophila suzukii) within unsorted trap catches. *Frontiers in Ecology and Evolution, 10*, 822648.

Radulovici, A. E., Archambault, P., & Dufresne, F. (2010). DNA barcodes for marine biodiversity: moving fast forward? *Diversity, 2*(4), 450-472.

Ratnasingham, S., & Hebert, P. D. (2007). bold: The Barcode of Life Data System

Almuthanna k. Alkaraki, Abrar Rashdan, Mohammad B. Alsliman, Omar Gammoh, Esam Qnais, Abdelrahim Alqudah, Alaa A. A. Aljabali (http://www.barcodinglife.org). *Mol Ecol Notes, 7*(3), 355-364. doi:10.1111/j.1471-8286.2007.01678.x

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). *Molecular ecology notes, 7*(3), 355-364.

Ratnasingham, S., Wei, C., Chan, D., Agda, J., Agda, J., Ballesteros-Mejia, L., . . . Manjunath, R. (2024). BOLD v4: A Centralized Bioinformatics Platform for DNA-Based Biodiversity Data. In *DNA Barcoding: Methods and Protocols* (pp. 403-441): Springer.

Ratnasingham, S., Wei, C., Chan, D., Agda, J., Agda, J., Ballesteros-Mejia, L., . . . Hebert, P. D. N. (2024). BOLD v4: A Centralized Bioinformatics Platform for DNA-Based Biodiversity Data. In R. DeSalle (Ed.), *DNA Barcoding: Methods and Protocols* (pp. 403-441). New York, NY: Springer US.

Raupach, M. J., Rulik, B., & Spelda, J. (2022). Surprisingly high genetic divergence of the mitochondrial DNA barcode fragment (COI) within Central European woodlice species (Crustacea, Isopoda, Oniscidea). *ZooKeys, 1082*, 103.

Ríos-Castro, R., Romero, A., Aranguren, R., Pallavicini, A., Banchi, E., Novoa, B., & Figueras, A. (2021). High-throughput sequencing of environmental DNA as a tool for monitoring eukaryotic communities and potential pathogens in a coastal upwelling ecosystem. *Frontiers in Veterinary Science, 8*, 765606.

Rodriques, S. G., Stickels, R. R., Goeva, A., Martin, C. A., Murray, E., Vanderburg, C. R., . . . Macosko, E. Z. (2019). Slide-seq: A scalable technology for measuring genome-wide expression at high spatial resolution. *Science, 363*(6434), 1463-1467.

Sahu, A., Kumar, N., Singh, C. P., & Singh, M. (2023). Environmental DNA (eDNA): Powerful technique for biodiversity conservation. *Journal for Nature Conservation, 71*, 126325.

Şapcı Selamoğlu, H. (2022). DNA barcoding of two narrow endemic plants; Astragalus argaeus and Astragalus stenosemioides from Mount Erciyes, Turkey. *Conservation Genetics Resources, 14*(1), 81-84.

Satam, H., Joshi, K., Mangrolia, U., Waghoo, S., Zaidi, G., Rawool, S., . . . Malonia, S. K. (2023). Next-Generation Sequencing Technology: Current Trends and Advancements. *Biology (Basel), 12*(7). doi:10.3390/biology12070997

Schindel, D. E., & Miller, S. E. (2005). DNA barcoding a useful tool for taxonomists. *Nature*.

Seberg, O., & Petersen, G. (2009). How many loci does it take to DNA barcode a crocus? *PloS one, 4*(2), e4598. doi:10.1371/journal.pone.0004598

Seifert, K. A., Samson, R. A., Dewaard, J. R., Houbraken, J., Lévesque, C. A., Moncalvo, J.-M., . . . Hebert, P. D. N. (2007). Prospects for fungus identification using CO1 DNA barcodes, with Penicillium as a test case. *Proceedings of the National Academy of Sciences, 104*(10), 3901-3906.

Shadrin, D. M. (2021). DNA barcoding: applications. *Russian Journal of Genetics, 57*, 489-497.

Shelton, A. O., Gold, Z. J., Jensen, A. J., D' Agnese, E., Andruszkiewicz Allan, E., Van Cise, A., . . . Parsons, K. (2023). Toward quantitative metabarcoding. *Ecology, 104*(2), e3906.

Sheth, B. P., & Thaker, V. S. (2017). DNA barcoding and traditional taxonomy: an integrated approach for biodiversity conservation. *Genome, 60*(7), 618-628. doi:10.1139/gen-2015-0167

Shokralla, S., Gibson, J. F., Nikbakht, H., Janzen, D. H., Hallwachs, W., & Hajibabaei, M. (2014). Next-generation DNA barcoding: using next-generation sequencing to enhance and accelerate DNA barcode capture from single specimens. *Molecular ecology resources, 14*(5), 892-901.

Shokralla, S., Hellberg, R. S., Handy, S. M., King, I., & Hajibabaei, M. (2015). A DNA mini-barcoding system for authentication of processed fish products. *Scientific reports, 5*(1), 15894.

Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular ecology, 21*(8), 1794-1805.

Shumskaya, M. (2024). DNA Barcoding for an Undergraduate Class. *Methods Mol Biol, 2744*, 537-550. doi:10.1007/978-1-0716-3581-0_33

Simaika, J. P., Stribling, J., Lento, J., Bruder, A., Poikane, S., Moretti, M. S., . . . Macadam, C. R. (2024). Towards harmonized standards for freshwater biodiversity monitoring and biological assessment using benthic macroinvertebrates. *Science of The Total Environment, 918*, 170360.

doi:https://doi.org/10.1016/j.scitotenv.2024.170360

Sohsah, G. N., Ibrahimzada, A. R., Ayaz, H., & Cakmak, A. (2020). Scalable classification of organisms into a taxonomy using hierarchical supervised learners. *J Bioinform Comput Biol, 18*(5), 2050026. doi:10.1142/s0219720020500262

Song, W., Zhang, T., Zhang, X., Warren, A., Song, W., Zhao, Y., & Luo, X. (2020). Taxonomy, Ontogenesis and Evolutionary Relationships of the Algae-Bearing Ciliate Bourlandella viridis () comb. nov., With Establishment of a New Genus and New Family (Protista, Ciliophora, Hypotrichia). *Front Microbiol, 11*, 560915. doi:10.3389/fmicb.2020.560915

Spooner, D. M. (2009). DNA barcoding will frequently fail in complicated groups: An example in wild potatoes. *Am J Bot, 96*(6), 1177-1189. doi:10.3732/ajb.0800246

Srivathsan, A., Nagarajan, N., & Meier, R. (2019). Boosting natural history research via metagenomic clean-up of crowdsourced feces. *PLoS biology, 17*(11), e3000517.

Stoeckle, M. Y., & Hebert, P. D. N. (2008). Barcode of life. *Scientific American, 299*(4), 82-89.

Stuart, T., Srivastava, A., Madad, S., Lareau, C. A., & Satija, R. (2021). Single-cell chromatin state analysis with Signac. *Nature methods, 18*(11), 1333-1341.

Sujeevan, R., & Hebert, P. (2007). BOLD: the barcode of life data system. *Molecular ecology notes, 7*(3), 355-364.

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular ecology, 21*(8), 2045-2050.

Thiele, K. R., Conix, S., Pyle, R. L., Barik, S. K., Christidis, L., Costello, M. J., . . . Garnett, S. T. (2021). Towards a global list of accepted species I. Why taxonomists sometimes disagree, and why this matters. *Organisms Diversity & Evolution, 21*(4), 615-622. doi:10.1007/s13127-021-00495-y

Tolley-Jordan, L. R., Chadwick, M. A., & Triplett, J. K. (2023). DNA barcoding indicates multiple invasions of the freshwater snail Melanoides tuberculata sensu lato in Florida. *PloS one, 18*(12), e0292164. doi:10.1371/journal.pone.0292164

Tsuji, S., Inui, R., Nakao, R., Miyazono, S., Saito, M., Kono, T., & Akamatsu, Y. (2022). Quantitative environmental DNA metabarcoding shows high potential as a novel approach to quantitatively assess fish community. *Scientific reports, 12*(1), 21524. doi:10.1038/s41598-022-25274-3

Valentini, A., Pompanon, F., & Taberlet, P. (2009). DNA barcoding for ecologists. *Trends in ecology & evolution, 24*(2), 110-117.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., . . . Boyer, F. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular ecology, 25*(4), 929-942.

Vences, M., Miralles, A., & DeSalle, R. (2024). A Glossary of DNA Barcoding Terms. *Methods Mol Biol, 2744*, 561-572. doi:10.1007/978-1-0716-3581-0_35

Vernooy, R., Haribabu, E., Ruiz Muller, M., Vogel, J., & Hebert, P. (2010). Barcoding Life to Conserve.

Vernygora, O. V., Sperling, F. A. H., & Dupuis, J. R. (2024). Toward transparent taxonomy: an interactive web-tool for evaluating competing taxonomic arrangements. *Cladistics, 40*(2), 181-191. doi:https://doi.org/10.1111/cla.12563

Vuataz, L., Reding, J. P., Reding, A., Roesti, C., Stoffel, C., Vinçon, G., & Gattolliat, J. L. (2024). A comprehensive DNA barcoding reference database for Plecoptera of Switzerland. *Sci Rep, 14*(1), 6322. doi:10.1038/s41598-024-56930-5

Wang, Z., Wang, T., Zhang, X., Wang, J., Yang, Y., Sun, Y., . . . Watson, A. E. (2024). Biodiversity conservation in the context of climate change: Facing challenges and management strategies. *Science of The Total Environment*, 173377.

Xu, J. (2016). Fungal DNA barcoding. *Genome, 59*(11), 913-932. doi:10.1139/gen-2016-0046

Yang, B., Zhang, Z., Yang, C. Q., Wang, Y., Orr, M. C., Wang, H., & Zhang, A. B. (2022). Identification of Species by Combining Molecular and Morphological Data Using Convolutional Neural Networks. *Syst Biol,*

Almuthanna k. Alkaraki, Abrar Rashdan, Mohammad B. Alsliman, Omar Gammoh, Esam Qnais, Abdelrahim Alqudah, Alaa A. A. Aljabali *71*(3), 690-705. doi:10.1093/sysbio/syab076

Yang, C. H., Wu, K. C., Chuang, L. Y., & Chang, H. W. (2022). DeepBarcoding: Deep Learning for Species Classification Using DNA Barcoding. *IEEE/ACM Trans Comput Biol Bioinform, 19*(4), 2158-2165. doi:10.1109/tcbb.2021.3056570

Yu, J., Wu, X., Liu, C., Newmaster, S., Ragupathy, S., & Kress, W. J. (2021). Progress in the use of DNA barcodes in the identification and classification of medicinal plants. *Ecotoxicol Environ Saf, 208*, 111691. doi:10.1016/j.ecoenv.2020.111691

Zhu, S., Liu, Q., Qiu, S., Dai, J., & Gao, X. (2022). DNA barcoding: an efficient technology to authenticate plant species of traditional Chinese medicine and recent advances. *Chin Med, 17*(1), 112. doi:10.1186/s13020-022-00655-y